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THERMAL INACTIVATION OF DIFFERENT MEASLES VIRUS STRAINS

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Summary. - The kinetics of thermal inactivation at 45 °C was investigated with different measles virus strains. The decrease of virus infectivity was determined by plaque counting test. The inactivation velocity of strains Schwarz, Moraten, Woodfalk, L16/SSW, L16/Moscow, and L16/S3 based on regression curve analysis (virus titres by time) allowed to divide them into 3 categories according to temperature sensitivity. Our results showed that development of a thermostable measles vaccine does not require selection of a thermoresistant virus variant if certain conditions for virus harvest are fulfilled.

Key words: measles vaccine; thermal inactivation; regression analysis

For the development of thermostable second generation measles vaccines (Colinet *et al.*, 1982) it is important to know the thermoresistance of corresponding viral strains. According to the WHO regulation the vaccine virus should not loose more than 1 log of its infectivity titre when kept for a week at 37 °C (WHO, 1981). In addition to following the technological schedule and using a suitable preservative, the choice of virus strain is the most important prerequisite for the production of a thermostable living virus vaccine.

Already in the 60-ties the T_{50} (thermosensitivity) marker was considered for determination of the degree of measles virus attenuation; this marker was associated with the virulence of the virus (Gordienko, 1973). We investigated the thermoresistance of different measles virus strains in the course of development of a thermostable measles vaccine. The results of such comparative investigations are presented here. For better and more precise estimations the slightly modified method of Albrecht and Schumacher (1972) was adopted to the treatment temperature of 45 °C.

Five vaccine strains (variants) and a nonattenuated strain were included into the investigations. All strains have been propagated in Vero cells. The infected cells were incubated at 32 °C or 35 °C in Eagle's MEM containing 2% calf serum. When about 75% of cells showed CPE the virus was harvested; already 12 - 24 hr before harvest the cells were kept in serum-free medium.

For each thermal inactivation of a virus strain the amount of virus in the specimens was determined after 0,20 and 90 min of thermal treatment (45 °C) using a plaque counting method in Vero

cells under carboxymethylcellulose overlay. Each virus was tested 3 - 4 times. The following calculations were made:

1. linear regression analysis of virus titres (PFU/ml to time t)
   $y = a - b \cdot t$
2. logarithmic expression of time dependence
   $y = a - b \cdot \ln (t + 1)$
3. exponential expression
   $y = a \cdot b^{(t+1)}$

In addition to the correlation coefficient (r), the time ($t_{y_0}-1$) could be determined which elapsed until the virus titre was altered by 1 log. This value was calculated for each strain using the 3 different equations (1, 2, 3), while the value chosen corresponded to the highest "r" coefficient, i. e. with the best data correlation to the given equation.

Strain L16/SSW was grown from a randomly selected commercial vaccine ampoule (Measles vaccine SSW^R Sächsisches Serumwerk, Dresden). The material was harvested at 50 % CPE, (lst harvest), at 75 % CPE (2nd harvest), and at 100% CPE (4st harvest). The virus designated L16/SSW/S3 is a variant from the vaccine strain. The vaccine strain "Moraten" was prepared from a single dose of vaccine "Attenuvax"$^{(R)}$ (Merck, Sharp, and Dohme, U. S. A.), strain "Schwarz" was derived from "Rimevax"$^{(R)}$ (Smith Kline, RIT Belgium). A clone with increased thermostability was derived from the U. S. S. R. test vaccine L16 (Gordienko, personal communication). The wild strain "Woodfalk" was kindly provided by Prof. ter Meulen (Würzburg, F. R. G.).

Table 1 shows the mean values of thermal inactivation of individual measles strains, which can be divided into 3 groups. To group I belongs strain L16/SSW from harvests 2 and 4, which are the most sensitive to thermoinactivation. Probably cell enzymes escaping to the medium at the high grade CPE may be the cause of the thermolable behaviour of the two materials from this strain. Group II comprises strain L16/SSW at the lst harvest, strains Moraten and Schwarz, all strains derived from worldwide used live vaccines. These strains behave alike concerning their $t_{y_0}-1$ values, the differences being insignificant. From the paper of Albrecht *et al.* (1972) values of 24.5 and 46.1 minutes can be calculated for strains Schwarz and Moraten, respectively, when correcting an apparent mistake in calculation of the inactivation constants by the authors.

Table 1. Time for reduction of virus titre of measles virus strains by 1 log PFU at 45 ^{0}C

Virus strain	$t_{y_0}-1$ (min)	calculated by formula	Group
L16/SSW, 4th harvest	5.14	(2)	I
L16/SSW, 2nd harvest	5.30	(2)	I
Schwarz	33.2	(3)	II
Moraten	49.9	(3)	II
L16/SSW, lst harvest	50.6	(3)	II
L16/SSW/S3	89.7	(1)	III
L16/SU/thermostable	92.3	(1)	III
Woodfalk	99.4	(3)	III

Their data then seem in accord with the values determined here. Group III contains the strains Woodfalk, L16/SU/thermostable and, surprisingly, also L16/SSW/S3. It is known that measles virus wild strains are highly thermostable (Cernescu *et al.*, 1984). With some likelyhood strain L16/SU/thermostable is supposed to be a clone selected from L16/SU and the behaviour of L16/SSW/S3 confirms the heterogeneity of the vaccine virus L16/SSW, a phenomenon known for L16 in general (Shteinberg *et al.*, 1977).

The distinction of 3 measles virus groups according to their thermostability is based on variation analysis showing an intergroup mean deviation (MD) of 34.99 min and an intragroup MD of 7.98 min. The differences in value $t_{y_0}-1$ are significant in the Scheffe-test. Only the differences between the harvests 2 and 4 of L16/SSW on one side and the strain Schwarz on the other was not significant probably due to a small number of tests. Selection of a highly thermoresistant variant has been shown not be an essential condition for obtaining a thermostable measles vaccine, if certain conditions are fulfilled in preparation of the virus material. The strain L16/SSW is more thermosensitive under the action of cellular enzymes, as its thermal behaviour depends on the degree of the CPE. The strain Schwarz form the thermostable Rimevax^R vaccine does not differ from other widely used measles vaccine strains. However, it should not be harvested from cells showing extensive CPE.

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References

Albrecht, P., and Schumacher, H. P. (1972): Markers for measles virus. I. Physical properties. *Arch. Virusforsch.* **36**, 23 - 35

Cernescu, C., and Cajal, N. (1984): Antimeasles vaccination by natural routes - experimental background and practical consequences. *Rev. Romm. Med. Virol.* **35**, 259 - 271.

Colinet, G., and Peetermans, J. (1982): Behaviour of five commercial measles vaccines in an accelerated stability test. *J. Biol. Standard.* **10**, 241 - 247.

Gordienko, N. M., Yakovleva, G. S., and Andzhaparidze, O. G. (1973): Changes in some genetic markers of measles virus strain L16 in the course of passaging in various cell cultures. *Acta virol.* **17**, 366.

Shteinberg, L. Sh., and Gordienko, N. M. (1977): Genetic characteristics of clones derived from measles virus strain L16. *Acta virol.* **21**, 383 - 390.

WHO (1981): Expanded programme of immunization. Stability of freeze-dried measles vaccine. *Wkly. Epidem. Rec.* **56**, 177 - 178.